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Original Contribution

Associations between Breast Cancer Risk and the Catalase Genotype, Fruit and Vegetable Consumption, and Supplement Use

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Observed weak or null associations between fruit and vegetable intake and breast cancer risk could be due to heterogeneity in endogenous antioxidant capabilities. The authors evaluated potential relations between a functional polymorphism in catalase, an antioxidant enzyme, and breast cancer risk, particularly in relation to fruit and vegetable intake and supplement use. Women (1,008 cases and 1,056 controls) in the Long Island Breast Cancer Study Project (1996–1997) were interviewed, completed a food frequency questionnaire, and provided blood for genotyping. The high-activity catalase *CC* genotype was associated with an overall 17% reduction in risk of breast cancer compared with having at least one variant *T* allele (odds ratio = 0.83, 95% confidence interval: 0.69, 1.00). Vegetable and, particularly, fruit consumption contributed to the decreased risk associated with the catalase *CC* genotype. Associations were more pronounced among women who did not use vitamin supplements, with a significant multiplicative interaction ($p_{interaction} = 0.02$) for the *CC* genotype and high fruit intake (odds ratio = 0.59, 95% confidence interval: 0.38, 0.89), and there was no association among supplement users. These results indicate the importance of diet, rather than supplement use, in concert with endogenous antioxidant capabilities, in the reduction of breast cancer risk. *CC* genotypes were prevalent in approximately 64% of controls; thus, the preventive potential for fruit consumption has widespread implications.

breast neoplasms; catalase; fruit; oxidative stress; vegetables; vitamins

Abbreviations: *CAT*, catalase (gene); CI, confidence interval; LIBCSP, Long Island Breast Cancer Study Project; OR, odds ratio.

Several recent cohort studies have noted no associations between fruit and vegetable consumption and risk of breast (1, 2) and total (3) cancers. However, it is plausible that fruit and vegetable consumption, particularly dietary sources of antioxidants, may interact with endogenous sources of pro- and antioxidants, and that such consumption may modify the effects of genetic factors related to oxidative stress, thereby impacting breast cancer risk. These potential effects are

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important for public health, in that fruit and vegetable consumption may ameliorate the increased risk associated with certain genetic polymorphisms or potentiate the inverse associations with other variants in a multiplicative manner.

Oxidative stress clearly has a role in carcinogenesis (4). Reactive oxygen species can cause oxidative damage to biomolecules (e.g., DNA), and multiple antioxidant defenses can neutralize reactive oxygen species (5–8). Variability in exposure to factors that could affect the levels of reactive oxygen species, through endogenous processes or exogenous routes, will ultimately impact levels of oxidative stress. Oxidative damage has been reported to be higher in women with breast cancer, compared with controls (9, 10), and, as reviewed by Ambrosone (11), could be involved in breast cancer etiology.

The catalase enzyme is an endogenous antioxidant enzyme that neutralizes reactive oxygen species by converting H_2O_2 into H_2O and O_2. Together with other antioxidant enzymes, including superoxide dismutase and glutathione peroxidase, catalase is a primary defense against oxidative stress. Acatalasemic mice, with approximately one tenth of the catalase blood and tissue levels of normal mice, are more susceptible to mammary carcinoma than their counterparts with normal levels are (12). A common catalase-262 $C \rightarrow T$ polymorphism has been identified in the promoter region of the human catalase gene (*CAT*), and it is plausible that the endogenous variability associated with this polymorphism plays a role in the host response to oxidative stress.

Because of the support for the role of oxidative stress in breast cancer etiology (11) and the importance of catalase in neutralizing reactive oxygen species, it is plausible to hypothesize that *CAT* polymorphisms (reference single nucleotide polymorphism (rs#) 1001179) may influence breast cancer risk. Because data on the functional effects of the catalase-262 $C \rightarrow T$ polymorphism are limited, we first evaluated associations between this genotype and catalase activity in red blood cells from a small sample of volunteers. We also evaluated the association between the *CAT* polymorphism and the risk of breast cancer and assessed potential modifying influences of fruit and vegetable intakes and specific antioxidant supplement use on risk relations in the Long Island Breast Cancer Study Project (LIBCSP).

MATERIALS AND METHODS

The LIBCSP study population

The LIBCSP, a population-based case-control study of breast cancer, was described previously (13). Briefly, cases were English-speaking women over 20 years of age with newly diagnosed, primary in situ or invasive breast cancer who resided in Nassau and Suffolk counties in Long Island, New York (1996–1997). Women with breast cancer were identified through regular contact with the pathology departments of the 31 institutions in the Long Island-New York City area, and the diagnosis was verified by the treating physician. English-speaking controls, matched to the expected age distribution of cases by 5-year age groups, were identified by use of Waksberg's method of random digit dialing (14) for women under the age of 65 years and by the rosters of the Center for Medicare and Medicaid Services (formerly Heath Care Financing Administration) for women who were 65 years or older. All respondents provided signed, informed consent prior to the study interview.

Upon receipt of physician and participant consents, 1,508 cases (82.1 percent) and 1,556 controls (62.8 percent) were interviewed in their homes by a trained interviewer. Among case and control respondents who completed the interviewer-administered questionnaire, 98.2 and 97.6 percent self-completed the food frequency questionnaire, and 73.0 and 73.3 percent donated a blood sample (13), respectively. As published previously (13), the relations between breast cancer risk and traditional risk factors, including lower parity, late age at first birth, little or no breastfeeding, family history of breast cancer, and increasing income and education, were observed. Results were similar when the analyses were restricted to respondents who donated blood (13) or to those with DNA available for these analyses (data not shown). Case-control status and fruit and vegetable consumption were not predictors of blood donation. Among those for whom DNA was available, 94 percent of cases and 93 percent of controls were Caucasian. The age ranges of cases and controls were 25.1–98.1 (mean: 58.6) years and 20.3–95.5 (mean: 56.1) years, respectively.

Measurements

Catalase activity by genotype among 18 volunteers. Subjects. Blood samples were obtained from healthy volunteers ($n = 18$) at Roswell Park Cancer Institute, Buffalo, New York. All participants for the catalase activity study were Caucasian and primarily female (80 percent), ranging in age from 20 to 64 years.

Activity measurement by *CAT* genotype. Erythrocytes were separated by centrifugation, and catalase activity was measured using a commercially available kit (Oxis Research, Portland, Oregon). Briefly, 30 μl of diluted erythrocyte lysate were incubated with 10 mM H_2O_2 at room temperature for exactly 1 minute. An aliquot was added to horseradish peroxidase/chromagen reagent and allowed to develop for 10 minutes. Absorbance was read at 520 nm, and activity units were assigned on the basis of a calibration curve of known amounts of H_2O_2. Activity was normalized to erythrocyte counts. The *CAT* genotype was determined by direct sequencing using the Beckman CEQ genetic analysis system (Beckman Coulter, Inc., Fullerton, California).

CAT genotyping among LIBCSP participants. Genomic DNA was extracted from mononuclear cells in whole blood separated by Ficoll (Sigma Chemical Co., St. Louis, Missouri). Pelleted cells were frozen at −80°C until DNA isolation by standard phenol, and chloroform isoamyl alcohol extraction and RNase treatment were performed (15). Genotyping was performed by BioServe Biotechnologies (Laurel, Maryland) by use of the high-throughput, matrix-assisted, laser desorption/ionization time-of-flight mass spectrometry of Sequenom, Inc. (San Diego, California), as previously described (16), using the primers 5′-ACGTTGGATGTCTGGCCCAGCAATTGGAGAG-3′ and 5′-CGTTGGATGAGGATGCTGATAACCGGGAG-3′. All

genotyping results were reviewed manually for quality control. Controls for genotype and two nontemplate controls were included on each plate. In addition, 170 sets of blinded controls (8 percent) were distributed throughout the DNA samples for quality control purposes. Laboratory personnel were blinded to case/control status. *CAT* genotype data were available for 1,017 cases and 1,071 controls.

Other exposure assessment among LIBCSP participants. The questionnaire focused on known and suspected risk factors for breast cancer, including reproductive, hormonal, medical, and lifestyle histories. For assessment of diet for the 12 months prior to the interview, 98 percent of participants completed a self-administered, modified National Cancer Institute–Block food frequency questionnaire, which was validated previously (17). The food frequency questionnaire included questions related to 13 fruits and fruit juices and 16 vegetables (excluding French fries), in addition to questions about supplement use (multiple vitamins and single supplements of vitamins A, E, and C, as well as β-carotene). Frequency and portion size data were translated to daily nutrient intakes by use of the National Cancer Institute's DietSys, version 3 (18). Participants with daily energy intakes above or below 3 standard deviations of the log-transformed mean (cases = 9, controls = 15) were dropped from the analyses (19).

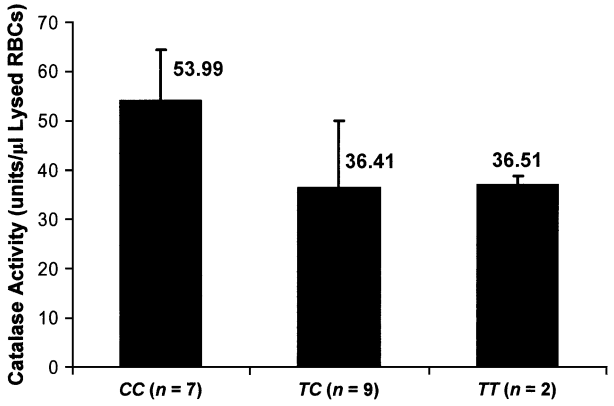

FIGURE 1. Mean catalase activity (with standard deviation) in red blood cells by catalase-262 *CC, TC,* and *TT* genotypes from specimens collected at Roswell Park Cancer Institute, Buffalo, New York, 2004. Subjects with the *CC* genotype had significantly higher catalase activity than did those with the *TC* or *TT* genotype ($p = 0.028$ among *CC, TC,* and *TT* genotypes; $p = 0.007$ between *CC* and *TC* and *TT* combined, by analysis of variance and Student's *t* tests). RBCs, red blood cells.

Statistical analysis

Among the 18 volunteers, levels of catalase activity were compared with *CAT* genotypes by use of one-way analysis of variance and Student's *t* tests. The measure of observer agreement of genotype between 8 percent of randomly selected duplicates that were included for quality control purposes was assessed using the kappa statistic. Among the 1,008 cases and 1,056 controls from the LIBCSP, unconditional logistic regression (20) was used to calculate odds ratios and corresponding 95 percent confidence intervals for breast cancer, in relation to genotype. Tests for Hardy-Weinberg equilibrium among the controls were conducted by use of observed genotype frequencies and a χ^2 test with 1 df. Multivariate models were adjusted simultaneously for age at reference date (defined as the date of diagnosis for cases and the date of identification for controls), family history of breast cancer (first-degree relative), and body mass index at reference date. The other variables assessed did not confound the associations of interest. The final multivariate models shown include only the factors that changed the estimated effect by 10 percent or more, which was developed by starting with a full model and then excluding covariates that did not improve the overall fit, as measured by the −2 log-likelihood ratio test (20). Total caloric intake was included in the multivariate model to control for confounding by total energy intake (21).

For dietary factors, fruit and vegetable consumption and specific dietary antioxidants were dichotomized into categories of the lowest three quintiles versus the highest two quintiles, on the basis of the distributions in controls of each factor. This categorization was based on previous findings in the LIBCSP of the similarity of the odds ratios in each of those groups (18). Gene-environment interactions were evaluated by joint categories of *CAT* genotype, fruit and vegetable intake, and dietary antioxidants (vitamin C, vitamin E, or β-carotene). We evaluated modification of risk relations with genotype by use of dietary sources of antioxidants only and then by dietary antioxidants plus supplement sources. Variables for combined effects were coded using a common referent group (e.g., *CAT TC* and *TT* genotypes combined with lower category of dietary intake).

Because there is concern that use of supplements could modify associations among diet, genotypes, and breast cancer risk, we also stratified by supplement use (any of vitamin C, vitamin E, or β-carotene supplement). To test multiplicative interactions, we included a cross-product term of the ordinal score for each genotype and dietary antioxidant intake in multivariate models. The log-likelihood statistic for models that included a multiplicative interaction term was compared with that for models that did not. Tests for trend were conducted using the continuous values for dietary antioxidant intakes. All analyses were conducted using SAS, version 8.2, software (SAS Institute, Inc., Cary, North Carolina). All statistical tests were two sided.

RESULTS

CAT genotype and catalase activity in 18 volunteers

Associations between catalase activity and *CAT* genotypes are shown in figure 1. Volunteers with the common *CC* genotype ($n = 7$) had significantly higher catalase activity compared with those with either the *TC* or *TT* genotype ($p = 0.007$). No differences in catalase activity were

TABLE 1. Risk associated with catalase polymorphisms, Long Island Breast Cancer Study Project, 1996–1997

	Cases ($n = 1,008$)		Controls ($n = 1,056$)		Odds ratio*	95% confidence interval	Odds ratio†	95% confidence interval
	No.	%	No.	%				
Total participants								
TT	45	4.5	42	4.0	1.00		1.00	
TC	349	34.6	335	31.7	0.98	0.62, 1.53	0.95	0.58, 1.46
CC	614	60.9	679	64.3	0.85	0.55, 1.31	0.77	0.49, 1.22
TT and *TC*	394	39	377	36	1.00		1.00	
CC	614	61	679	64	0.86	0.72, 1.03	0.83	0.69, 1.00
Premenopausal women‡	323		356					
TT and *TC*	127	39	132	37	1.00		1.00	
CC	196	61	224	63	0.90	0.64, 1.22	0.84	0.61, 1.17
Postmenopausal women‡	661		657					
TT and *TC*	257	39	233	35	1.00		1.00	
CC	404	61	424	65	0.87	0.69, 1.08	0.83	0.66, 1.04

* Unconditional logistic regression, adjusted for age.
† Unconditional logistic regression, adjusted for age, family history, and body mass index.
‡ Excluding 67 subjects missing information on menopausal status.

observed between those heterozygous (*TC*, $n = 9$) and homozygous (*TT*, $n = 2$) for the variant allele. Results were the same when we compared catalase activity by genotype only among female participants (80 percent, data not shown).

CAT genotype and breast cancer risk in the LIBCSP

For genotyping in the LIBCSP, there was excellent agreement in the 8 percent of randomly selected duplicates that were included for quality control purposes (kappa statistic of 0.95 for discrepant results), with less than a 1 percent failure rate of the assay. Sensitivity analysis resulted in the same risk estimates. Genotype distribution followed Hardy-Weinberg equilibrium ($p = 0.85$) and was comparable with that of other published studies (22, 23).

Associations between *CAT* genotypes and breast cancer risk among women in the LIBCSP are shown in table 1. After adjustment for possible confounding factors (age, family history, and body mass index), the *CC* genotype, reflecting higher red blood cell catalase activity, was associated with a 17 percent reduction in breast cancer risk (multivariate odds ratio (OR) = 0.83, 95 percent confidence interval (CI): 0.69, 1.00; age-only adjusted OR = 0.86, 95 percent CI: 0.72, 1.03), compared with having the *TT* and *TC* genotypes combined. Estimates associated with *TC* genotypes did not differ from those for the *TT* genotype. Based on these data and the similarity in catalase red blood cell activity for those having any *T* alleles, further analyses were performed by combining *TC* and *TT* genotypes as the referent in the LIBCSP data set and contrasting *CC* genotypes against that group. The relations were similar between pre- and postmenopausal women (for multiplicative interaction: $p = 0.89$) and, thus, women were combined for all further analysis.

CAT genotype, fruit and vegetable consumption, dietary antioxidants, and breast cancer risk in the LIBCSP

The odds ratios for breast cancer risk by *CAT* genotype and fruit and vegetable consumption are shown in table 2. We observed a significant 29 percent reduction in risk (OR = 0.71, 95 percent CI: 0.54, 0.92) among women with the *CC* genotype and higher consumption of fruits (>10 servings/week), compared with those with at least one *T* allele (*TC* and *TT* combined) and lower fruit consumption levels. Associations between genotype and risk did not vary by vegetable consumption. Multiplicative interactions were not observed between *CAT* genotype and fruit and vegetable consumption in relation to breast cancer risk (for multiplicative interaction: $p = 0.33$, 0.51, and 0.58 for fruits only, vegetables only, and total fruits and vegetables).

We also evaluated specific dietary antioxidant intakes (i.e., vitamin C, vitamin E, and β-carotene) as possible active agents in the fruits and vegetables. We observed a statistically significant 26 percent reduction in risk among women with *CC* genotypes and a higher consumption of dietary vitamin C (>133.7 mg/day), compared with those with at least one *T* allele and lower consumption (≤133.7 mg/day) (OR = 0.74, 95 percent CI: 0.57, 0.97). This pattern was also observed for dietary vitamin E, albeit to a lesser degree. However, for β-carotene, reduced risk was also noted for high consumers with *T* alleles, although this was of borderline significance (OR = 0.74, 95 percent CI: 0.55, 1.00). We also evaluated associations with the total contribution of dietary and supplement sources; results were similar. The total intake of antioxidants including supplements was not associated with any additional risk reduction beyond that conferred by dietary sources alone.

TABLE 2. Risk associated with catalase polymorphisms by low and high intake of fruits and vegetables and other antioxidants, Long Island Breast Cancer Study Project, 1996–1997

	TT and *TC* genotypes				*CC* genotype			
	Cases (no.)	Controls (no.)	Odds ratio*	95% confidence interval	Cases (no.)	Controls (no.)	Odds ratio*	95% confidence interval
Fruits†								
≤10 servings/week	223	211	1.00		365	380	0.87	0.68, 1.11
>10 servings/week	171	166	0.90	0.67, 1.21	249	299	0.71	0.54, 0.92
Vegetables								
≤16 servings/week	233	211	1.00		350	388	0.78	0.61, 0.99
>16 servings/week	157	162	0.88	0.65, 1.18	256	283	0.80	0.61, 1.04
Fruits and vegetables								
≤33 servings/week	244	214	1.00		382	404	0.81	0.64, 1.03
>33 servings/week	150	163	0.80	0.59, 1.08	232	275	0.69	0.53, 0.90
Vitamin C								
From food only								
≤133.7 mg/day	236	227	1.00		403	411	0.91	0.72, 1.15
>133.7 mg/day	158	150	1.03	0.76, 1.40	211	268	0.74	0.57, 0.97
From food and supplement								
≤210.1 mg/day	240	221	1.00		383	414	0.82	0.65, 1.04
>210.1 mg/day	154	156	0.93	0.69, 1.25	231	265	0.79	0.61, 1.03
Vitamin E								
From food only								
≤7.87 α-tocopherol equivalents/day	251	244	1.00		381	394	0.93	0.73, 1.17
>7.87 α-tocopherol equivalents/day	143	133	1.16	0.83, 1.62	233	285	0.81	0.61, 1.08
From food and supplement								
≤25.45 α-tocopherol equivalents/day	240	231	1.00		372	415	0.82	0.65, 1.04
>25.45 α-tocopherol equivalents/day	154	146	1.00	0.75, 1.25	242	264	0.86	0.66, 1.11
β-carotene								
From food only								
≤2,673 μg/day	250	216	1.00		384	408	0.77	0.61, 0.98
>2,673 μg/day	144	161	0.74	0.55, 1.00	230	271	0.69	0.53, 0.91
From food and supplement								
≤3,152.48 μg/day	260	211	1.00		375	412	0.71	0.56, 0.89
>3,152.48 μg/day	134	166	0.66	0.49, 0.89	239	267	0.72	0.55, 0.93

* Odds ratios and 95% confidence intervals calculated by unconditional logistic regression, adjusted for age, family history, body mass index, and total calories.

† Fruit and vegetable and other antioxidant consumption based on the lowest three-fifth and the highest two-fifth values of the control group.

Risk associations by supplement use in the LIBCSP

Since antioxidant vitamin supplements may modify risk through an oxidative stress mechanism, we also evaluated the effects of supplement use on modifying risk relations. Gaudet et al. (18) previously reported that antioxidant supplements were not associated with risk of breast cancer in the LIBCSP (OR = 0.93, 95 percent CI: 0.78, 1.11). Although there was no effect of supplement use on the relations between *CAT* genotypes and breast cancer risk, supplement use *did* modify the associations among risk, *CAT* genotype, and fruit and vegetable intake. As shown in table 3, inverse associations among *CAT* genotypes, high fruit and vegetable consumption, and breast cancer risk appeared to be strongest among women who did not use vitamin supplements. Among non-vitamin supplement users, we observed a statistically significant multiplicative interaction ($p = 0.02$), with the lowest risk observed for women who were high fruit consumers (>10 servings/week) and who had *CC* genotypes (OR = 0.59, 95 percent CI: 0.38, 0.89). Among supplement users, however, there was no significant multiplicative interaction between fruit consumption

TABLE 3. Risk associated with catalase polymorphisms among vitamin supplement users and non-supplement users consuming diets low and high in fruits and vegetables and other antioxidants, Long Island Breast Cancer Study Project, 1996–1997

	TT and *TC* genotypes				*CC* genotype				
	Cases (no.)	Controls (no.)	Odds ratio†	95% confidence interval	Cases (no.)	Controls (no.)	Odds ratio†	95% confidence interval	$p_{interaction}$*
Fruits‡									
Non-supplement user (cases/controls = 406/406)									
≤10 servings/week	99	92	1.00		167	153	0.94	0.65, 1.37	0.02
>10 servings/week	61	49	1.06	0.66, 1.73	79	112	0.59	0.38, 0.89	
Supplement user§ (cases/controls = 602/650)									
≤10 servings/week	124	119	0.97	0.66, 1.44	198	227	0.80	0.56, 1.13	0.62
>10 servings/week	110	117	0.81	0.54, 1.21	170	187	0.76	0.53, 1.10	
Vegetables									
Non-supplement user (cases/controls = 406/406)									
≤16 servings/week	103	89	1.00		157	165	0.74	0.51, 1.07	0.69
>16 servings/week	57	52	0.84	0.52, 1.38	89	100	0.70	0.46, 1.06	
Supplement user (case/controls = 602/650)									
≤16 servings/week	134	126	0.85	0.58, 1.25	201	231	0.69	0.48, 0.98	0.58
>16 servings/week	100	110	0.78	0.52, 1.17	167	183	0.74	0.52, 1.07	
Fruits and vegetables									
Non-supplement user (cases/controls = 406/406)									
≤33 servings/week	109	94	1.00		177	172	0.81	0.57, 1.16	0.22
>33 servings/week	51	47	0.81	0.49, 1.34	69	93	0.55	0.35, 0.85	
Supplement user (cases/controls = 602/650)									
≤33 servings/week	135	120	0.90	0.62, 1.31	205	232	0.73	0.52, 1.02	0.85
>33 servings/week	99	116	0.72	0.48, 1.08	163	182	0.70	0.49, 1.01	
Vitamin C									
Non-supplement user (cases/controls = 418/421)									
≤133.7 mg/day	108	98	1.00		187	179	0.90	0.63, 1.27	0.03
>133.7 mg/day	55	46	1.12	0.68, 1.84	68	98	0.62	0.40, 0.95	
Supplement user (cases/controls = 590/635)									
≤133.7 mg/day	128	129	0.90	0.61, 1.33	216	232	0.83	0.59, 1.18	0.66
>133.7 mg/day	103	104	0.91	0.60, 1.38	143	170	0.75	0.51, 1.09	

Table continues

and *CAT* genotype ($p = 0.62$). Nonetheless, point estimates of risk were consistently lower among women with *CC* genotypes regardless of supplement use.

Similar associations were noted for specific dietary antioxidant nutrients. Among non-supplement users, there was a statistical interaction ($p = 0.03$), with the lowest risk observed for high dietary vitamin C consumption and *CC* genotypes (OR = 0.62, 95 percent CI: 0.40, 0.95). An inverse linear trend between fruits and vitamin C (both continuous) and breast cancer risk appeared stronger among women with *CC* genotypes ($p_{trend} = 0.03$ and 0.03 for fruits and vitamin C, respectively) than among women with at least one *T* allele ($p_{trend} = 0.49$ and 0.35 for fruits and vitamin C). Among vitamin supplement users, inverse associations with vitamin C consumption and *CC* genotypes were less pronounced. However, even among supplement users, the lowest risk was observed in the group with *CC* genotypes and high dietary antioxidant intake. With regard to β-carotene, there were significant reductions in risk with high dietary consumption among both supplement users and nonusers. Among those taking β-carotene supplements, higher dietary intake was associated

TABLE 3. Continued

	TT and *TC* genotypes				*CC* genotype				$p_{interaction}$
	Cases (no.)	Controls (no.)	Odds ratio†	95% confidence interval	Cases (no.)	Controls (no.)	Odds ratio†	95% confidence interval	
Vitamin E									
Non-supplement user (cases/controls = 419/420)									
≤7.87 α-tocopherol equivalents/day	108	98	1.00		167	156	0.92	0.65, 1.32	0.05
>7.87 α-tocopherol equivalents/day	60	49	1.24	0.75, 2.05	84	117	0.66	0.43, 1.00	
Supplement user (cases/controls = 589/636)									
≤7.87 α-tocopherol equivalents/day	143	146	0.84	0.58, 1.24	214	238	0.78	0.55, 1.12	0.56
>7.87 α-tocopherol equivalents/day	83	84	0.93	0.59, 1.47	149	168	0.79	0.53, 1.17	
β-carotene									
Non-supplement user (cases/controls = 493/493)									
≤2,673 μg/day	133	102	1.00		200	200	0.72	0.52, 1.00	0.87
>2,673 μg/day	65	65	0.78	0.50, 1.22	95	126	0.56	0.38, 0.82	
Supplement user (cases/controls = 515/563)									
≤2,673 μg/day	117	114	0.79	0.54, 1.16	184	208	0.66	0.47, 0.92	0.09
>2,673 μg/day	79	96	0.58	0.38, 0.88	135	145	0.67	0.46, 0.97	

* To test multiplicative interactions, a cross-product term of the ordinal score for each genotype and dietary antioxidant intake was included in multivariate models. The log-likelihood statistic for models that included a multiplicative interaction term was compared with the statistic for those that did not.

† Odds ratios and 95% confidence intervals calculated by unconditional logistic regression, adjusted for age, family history, body mass index, and total calories.

‡ Fruit and vegetable and other antioxidant consumption based on the lowest three-fifth and the highest two-fifth values of the control group.

§ Supplement user: For fruit, vegetable, and fruit and vegetable groups, a supplement user was a woman taking any vitamin C, vitamin E, or β-carotene supplement. For vitamin C, vitamin E, and β-carotene, a supplement user was a woman taking only that specific vitamin supplement. Thus, the numbers in the cells are not the same.

with decreased risk among women with *TT* and *TC* genotypes, as well as *CC* genotypes.

DISCUSSION

In this large population-based case-control study, we found that the *CAT CC* genotype was associated with a 17 percent reduction in risk of breast cancer compared with having at least one variant *T* allele. More importantly, the interaction between the ''low-risk'' allele and higher consumption of fruits or dietary sources of vitamin C appeared to be apparent among women who did not use antioxidant vitamin supplements. These findings support the hypothesis that women with genotypes resulting in higher activity toward neutralization of reactive oxygen species have reduced risk of breast cancer and that higher consumption of vegetables and, particularly, fruits enhances the protective effects.

Although our study is the first to evaluate the *CAT* polymorphism in relation to cancer risk, it is plausible that the enzyme could play a role in cancer etiology. Malignant lung tumors have significantly decreased catalase activity (24). The phenotyping data reported here verified that the *CC* genotype was, indeed, associated with significantly higher enzyme activity in red blood cells. Previously, Forsberg et al. (25) showed that the *T* allele was associated with greater transcriptional activation in K-562 and HepG2 cell lines, but not in HeLa cell lines. They also found that the *T* allele was associated with higher catalase protein levels in blood ($n = 29$). This finding is in contrast to ours, in which the *CC* genotype was associated with higher enzyme activity in red blood cells. Differences are possibly due to the measurement of protein versus activity in red blood cells, or they reflect a dependence of the enzyme's stability on sample preparation methods. In both studies, the numbers of samples examined were quite small. Larger genotype-phenotype association studies are required before definitive conclusions can be reached. Nonetheless, several other studies support our observation that activity is reduced in carriers of *T* alleles. Ahsan et al. (22) showed that arsenic exposure resulted in hyperkeratosis to a much greater extent (fourfold) among those with *TT* genotypes

than among those with *CC* genotypes. The *TT* genotype also was associated with higher blood pressure compared with the *CC* genotype (26). Our results, showing that the *CC* genotype was associated with decreased risk, are consistent with these previous findings, and our findings for *CAT* also support an oxidative stress mechanism in breast cancer etiology.

In the LIBCSP, higher fruit and vegetable consumption was associated with decreased breast cancer risk among postmenopausal women, with weaker associations among premenopausal women (18). In this analysis, when intakes of fruits and vegetables and of specific dietary antioxidants were categorized by low and high consumption, risk reduction appeared greatest among women with *CC* genotypes who consumed higher amounts of vegetables and, particularly, fruits. These findings and the mechanism indicated are consistent with our earlier findings, that the *MPO* variant related to reduced generation of reactive oxygen species was associated with reduced breast cancer risk, particularly among women who consumed higher amounts of fruits and vegetables and of other specific dietary antioxidants (16). Similarly, in the Western New York Diet Study (27), we found that the increased risk associated with a variant form of the gene for manganese superoxide dismutase (*MnSOD*), an enzyme-neutralizing reactive oxygen species, was reduced among higher consumers of fruits, vegetables, and dietary antioxidants.

The mechanisms whereby *CAT* effects may be greatest for higher consumers of fruits are unclear. Fruit contains numerous putative anticarcinogenic substances and other nonnutrient antioxidants, such as terpenes, flavonoids (quercetin), and polyphenols, as well as vitamin C. It is possible that those chemopreventive components in fruits enhance the antioxidant capabilities of catalase, an effect that is not observed with antioxidant vitamin supplements alone. An in vitro study showed that the levels of *CAT* mRNA expression increased about 30 percent in quercetin-treated hepatoma cells, whereas the expression of other antioxidant enzymes (e.g., manganese superoxide dismutase and glutathione peroxidase) remained unchanged (28). Furthermore, treatment of rats with flavonoids prior to the administration of an iron chelate prevented the reduction of catalase enzyme activity and an increase in oxidative stress markers (29), indicating that the function of endogenous antioxidant systems may be maximized at high levels of the putative anticarcinogenic substances contained in fruits.

The weaker association between vitamin E and risk reduction may, in part, reflect that most dietary vitamin E is obtained from vegetable oils used in cooking, rather than from the consumption of vegetables (30). Intake of such oils is not estimated well by food frequency questionnaires (31). In addition, β-carotene consumption, unlike intakes of vitamins C and E, was associated with risk reduction in participants with either *T* alleles or the *CC* genotype. However, the biologic basis for this association is unclear. It is possible that the association is independent of the functions of catalase, implying a mechanism other than antioxidant properties for the putative benefits associated with β-carotene. It is also possible that the effects of dietary β-carotene are so strong that they are not impacted by *CAT* genotypes.

We observed a statistically significant interaction between *CAT* genotypes and fruit intake only among non-supplement users, although there was a modest risk reduction associated with supplement use overall among women with *CC* genotypes. Supplement users generally have higher dietary intakes of fruits and vegetables than non-supplement users do, and it is possible that intake from supplements overwhelms dietary intake, so that the latter has little impact on risk reduction. Thus, the small functional effect of the genotype could be overcome by a substantially increased intake of antioxidants. Alternatively, because the greatest risk reduction was observed among non-supplement users with high fruit consumption and *CC* genotypes, and because the additional antioxidants supplied by supplements did not significantly reduce cancer risk, it is also possible that other putative chemopreventive components in fruits, rather than specific vitamins, could account for the interaction between fruit intake and *CAT* genotype, as discussed above. This observation emphasizes the importance of dietary fruit and vegetable consumption. This is consistent with findings from the Nurses' Health Study showing stronger inverse associations between fruit and vegetable intakes and cancer among nonvitamin users than among multivitamin users (3). Considering the fact that 46 percent of women in the cohort used vitamin supplements (3), it may at least in part explain the lack of associations between fruit and vegetable consumption and the risk of breast cancer in that study.

Results from analyses of the LIBCSP data could also be affected by sources of bias that are common to case-control studies (e.g., selection bias or recall bias) (32) or by misclassification bias related to genotyping. However, problems with recalling the details of past exposures would be less likely to affect genotyping status, although diet and other interview data may be susceptible to this bias. Furthermore, the possible nondifferential misclassification bias due to diet and vitamin supplement use likely deflates the estimated odds ratio toward the null and, thus, true odds ratios could be larger than those observed (33, 34). Finally, in light of the contradictory data on the functional impact of the variant and the marginally significant odds ratios, the results may also be attributable to chance.

Our findings indicate that consumption of fruits and vegetables modifies endogenous oxidant and antioxidant capabilities and may impact breast cancer risk through gene/diet interactions. It is interesting to note that, although no associations were observed between consumption of fruits and vegetables and breast cancer risk in the Nurses' Health Study, a report evaluating serum antioxidants in that same study showed that carotenoids were inversely associated with breast cancer risk (35).

Fruits and vegetables are rich sources of these and other putative chemopreventive substances that may enhance the effectiveness of endogenous antioxidant enzymes. Although there is support for a role for oxidative stress in breast cancer, the results of epidemiologic studies of fruit and vegetable consumption have been inconsistent (21, 36–38). Allelic variability in genes that protect from oxidative stress could modify associations between fruits and vegetables and breast cancer risk and explain, in part, inconsistencies in the published literature, although several issues may contribute

to the null results, such as dietary measurement error and a short follow-up period in those cohorts (39, 40).

In summary, the high-activity *CAT CC* genotype was associated with a modest reduction in breast cancer risk. More importantly, higher consumption of fruits further decreased the inverse associations between *CAT* genotype and breast cancer risk. Our data do not support the view that supplements may enhance the inverse associations with high fruit or high fruit and vegetable intakes. We believe that the results could be interpreted to show that foods, rather than vitamins, are likely sources of numerous other risk-reducing properties and should be relied upon, rather than supplements, for health promotion.

To our knowledge, this is the first study to evaluate functional *CAT* genotypes and breast cancer risk. The study benefits from the large population-based sample with adequate statistical power and in-depth interview assessments. These data provide further support for a link between oxidative stress and breast cancer, and they contribute to a better understanding of the role of fruit intake in breast cancer risk, taking into consideration variability in endogenous antioxidant capabilities. Although the genotype may not be changed, it is encouraging to note that the inverse associations with *CAT* polymorphisms were observed primarily in women who consumed more fruits, particularly among nonvitamin supplement users. These findings underscore public health recommendations for the consumption of diets rich in fruits as well as vegetables as a means of cancer prevention.

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